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Effects of Bee Bread on Male Reproductive System of Sprague Dawley Rat

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ABSTRACT

Bee bread has been traditionally consumed for enhancement of male fertility. There has been an increasing demand for natural products, particularly the bee products. However, to date, the role of bee bread and its underlying potential mechanism for protecting or improving sperm parameters has not been reported. Therefore, this study determines the role of bee bread supplementation on male reproductive organ weight and sperm parameter in adult male rat. There are two groups which consist of treated and control group with 6 male rats in each group. The control rats were administered with 1ml of distilled water, while the treated rats were administered with 0.5g/kg body weight of bee bread (diluted in 1 ml of distilled water) for 28 days. At the end of experiment, rats were euthanised, and reproductive organs weight were collected and weighed. Cauda epididymis was collected for sperm count and sperm morphology evaluations. Apart from the prostate gland, there is no significant difference observed between the mean weight of testis, seminal vesicle, epididymis of the control rat and the treated. In addition, there is no significant difference in sperm count and sperm morphology between the control group and treated group. The results of this study show that bee bread supplementation causes an increase in the weight of the prostate gland in adult rats.

Keywords: Androgenic effect, bee bread, male fertility, male reproductive system, rat

ABSTRAK

Roti lebah telah digunakan secara tradisional untuk meningkatkan kesuburan lelaki. Terdapat permintaan yang semakin meningkat untuk produk semula jadi, terutamanya produk lebah. Bagaimanapun, setakat ini peranan roti lebah dan mekanisme potensinya untuk melindungi atau meningkatkan parameter sperma masih tidak dilaporkan. Oleh itu, kajian ini menentukan peranan pemberian roti lebah kepada berat badan, organ reproduktif dan parameter sperma pada tikus jantan dewasa. Terdapat dua kumpulan iaitu kumpulan rawatan dan kawalan yang terdiri daripada 6 tikus jantan dalam setiap kumpulan. Tikus kawalan telah diberikan dengan 1 ml air suling sementara tikus yang dirawat telah diberikan pada 0.5 g / kg berat badan roti lebah yang dicampurkan dalam 1 ml air suling selama 28 hari. Bahagian kauda epididimis diambil dan digunakan dalam evaluasi bilangan sperma dan mofologi sperma. Selain daripada kelenjar prostat, tiada perbezaan yang signifikan antara berat testis, vesikel semen, epididimis tikus kawalan dan tikus yang dirawat. Di samping itu, tiada perbezaan yang signifikan dalam bilangan sperma dan morfologi sperma antara kumpulan kawalan dan kumpulan tikus yang dirawat. Keputusan kajian ini menunjukkan bahawa pemberian roti lebah menyebabkan peningkatan berat kelenjar prostat pada tikus dewasa.

Kata Kunci: Kesan androgenik, roti lebah, kesuburan lelaki, sistem pembiakan jantan, tikus

INTRODUCTION

Bee bread produced from bee pollen that acts as raw material. Vialli (2014) reported that bees collect two principle of food which is nectar and pollen. First, bees collect nectar to make an enzymatically-activated, partially fermented food called honey. Second, they also collect pollen to make a lacto-fermented, enzymatically-activated food called bee bread. Honey is for bee primary carbohydrate source, and bee bread is usually being their staple protein source. Bee bread ingredient is an "alchemical" bee creation made with around 25% honey or nectar, 70% pollen and bee saliva that alongside the honey/nectar which is a broad range of probiotic bacteria and yeasts inoculates into pollen. This is an essential activity and necessary to kickstart of fermentation and pre-digestion. Pollen is tightly packed by the in-hive bees into the comb-cells and intermix it with the other mentioned ingredients. The bee bread has been made after a few weeks a great transformation has taken place. According to Couto and Couto (2006) and Pereira et al., (2006), bee bread undergoes anaerobic fermentation and is preserved by the arising lactic acid. The function of bee bread is to serve as the basic protein source for the bee colony.

In recent years, there is growing demand for natural products in the human diet. One of these natural supplements is bee bread. Bee bread is made of pollen, which has been gathered by bees and mixed with its digestive enzymes, carried back to the hive and packed into pellets then preserved with a tiny bit of honey and bee wax. This mixture undergoes different chemical processes due to the action of different enzymes, microorganisms, moisture, and temperature. According to Nagai (2002), the higher activity of bee bread causes a good preservation due to the inhibition of moulds as well as microorganism growth.

Male infertility involves a complex aetiology. There are many factors contributing to male infertility including structural abnormality, hormonal imbalance, previous infection, environmental factor, immunological factor, genetic factor, systemic disease, erectile function, spermatogenic dysfunction, and idiopathic. The bee bread might have important role in improving and protecting the male reproductive system from infertility problems and it is important to understand its underlying mechanism.

The purpose of this research is to study the effect of bee bread to the male reproductive organs weight (testis, epididymis, prostate gland and seminal vesicle) and sperm parameters (sperm morphology, sperm count) in adult male rats. The positive findings or result from this study may provide a new knowledge to further a clinical study on the potential use of bee bread as a supplement to abnormal sperm parameter and fertility problems. Furthermore, this may help in reducing the economic burden of an individual with fertility problem as artificial reproductive technologies that are too expensive. The income of beekeepers could be increased as well as demand for the bee bread increase if the study is successful.

MATERIAL AND METHODS

Animal treatment

In this study, 12 adult male Sprague-Dawley rats aged 8-10 weeks were used as the animal model. Animals were maintained as per National guidelines and protocols. All animals were kept in clean cages and maintain in a controlled and well-ventilated animal room at 25 ± 2 °C with 12-h light/12-h dark cycles. Commercial pellet food and water ad libitum were supplied to all animals. The animals were randomly divided into 2 groups (n=6/group), control group and treatment group.

Before the treatment started, each rat was weighted. The dose of bee bread was calculated according to the intake in a human which is 10 – 30 g per day (0.17 – 0.50 g/kg/day) based on the body surface area normalization method. In this study, rats were given bee bread with the dose of 0.5 g/kg body weight/day by oral gavages once daily for 28 days. Meanwhile, distilled water was given to rats in control group.

The condition of the animals was observed and recorded daily. Body weights of rats were recorded daily. After 28 days of treatment, all animals were sacrificed. Testis, accessory sex organs which are epididymis, seminal vesicle and prostate gland were dissected out, trimmed off the attached tissue, and weighed. The results of the weight organs were recorded.

Sperm Analysis

For sperm count and sperm morphology evaluations, cauda epididymis was dissected and minced with scissors in 2 ml normal saline. Then, the mixture was filtered with 80 mm nylon mesh and mixed with 2 drops of Eosin Y. The mixture was left for 30 minutes. This mixture was used for sperm count and for morphology.

After 30 minutes the epididymal content was diluted 20 times with normal saline by using ratio 1:20. The dilution was dropped into Neubauer's chamber after placed cover slide above the chamber. The sperm suspension was count by counting in a hemocytometer. The hemocytometer was placed under the light microscope and viewed under ×400 magnification. The spermatozoa were counted from 5 chosen small squares out of 25 large squares. The sperm was counted by counting sperm's head in the square an on the line of the square. To get the concentration of the original sperm sample then should be multiplied by the dilution factor. The count was repeated 5 times for each rat to minimize the error (Haron et al., 2010).

Sperm morphology was determined by observing the thin smear of epididymal sperm sample under a light microscope. Firstly, 1 drop of the mixture of minced cauda epididymis was drop on the slide. The mixture was smeared across the slide by using another slide. After that, the slide was let to dry for 1 day. The number of abnormal sperm morphology was counted randomly within 200 sperm which was observed from the smear (Haron et al., 2010).

Data Analysis

Statistical analysis was carried out using IBM SPSS Statistics for Windows, Version 19 (International Business Machines Corporation, New Castle, NY, USA). All data were analysed using Independent Samples T-test and expressed as mean ± SEM. Statistical significance was accepted at $p < 0.05$.

RESULTS AND DISCUSSION

The results for reproductive organ weights and sperm parameters are presented in Table 1. Testis weight was lower in treated group compared to control group. However, the different was not statistically significant. Previous study has shown that Palestinian honey supplementation reduces mean weight of testis but it is not statistically significant (Abdul-Ghani et. al., 2008). Selmano□ lu et al., (2009) also found that there were no significant changes in absolute weights of testis when rats consuming pollen of three different plant sources (Trifolium spp., Raphanus spp. and Cistus spp.) at 60 mg/per animal/ per day over a 30-day period. Epididymis has an important function in reproductive, such as maturation of sperm surface antigens enabling fertilisation, storage of sperm prior to ejaculation, resorption of seminiferous tubule fluid and secretion of molecules into the fluid. Present study shows that the weight of epididymis in rats treated with bee bread was increased compared to control group. Nevertheless, it was not statistically significant. Abdul-Ghani et. al., (2008) has demonstrated that consumption of 5% honey for 20 days increased the relative weight of the epididymis. Other studies also reported the same finding whereby honey consumption increases the weight of epididymis (Morakinyo et. al., 2008; Oyelowo et al., 2014). The seminal vesicles or seminal glands, are a pair of simple tubular glands posteroinferior to the urinary bladder and they are located within the pelvis. They secrete fluid that partly composes the semen. The present result shows no significant differences in mean weight of seminal vesicle. This finding is supported by a study from Selmano□ lu et. al., (2009), the result also showed that there is no significant different in mean weight of seminal vesicle of rats when consuming pollen groups. Furthermore, Mahaneem et. al., (2007) also observed there is no significant difference for the relative weight of seminal vesicle among the groups when rats consuming Tualang Honey. According to Abdul-Ghani et al. (2008), consumption of 5% honey for 20 days had no significant differences in weight of seminal vesicle. Unlike other reproductive organs, prostate gland shows significant differences between treated group and control group. Supplementation of bee bread significantly increases the weight of prostate gland compared to control group. Prostate gland is a compound tubuloalveolar exocrine gland of the male reproductive system. The function of the prostate is to secrete an alkaline fluid, milky or white in appearance, that constitutes 30% of the volume of the semen along with spermatozoa and seminal vesicle fluid. Bee products has been shown to possess an ability to inhibit tumour growth in vitro and in vivo (Premratanachai and Chanchao, 2014). Another study shows that bee pollen has a strong cytotoxicity effect on prostate cancer cells (PC-3 Cells) (Wu and Lou, 2007). They also reported that no significant toxic effect was observed either in biochemical analysis or histological investigation in rats given three different pollens. Blanc (2017) reported that bee pollen may be an effective complementary prevention and treatment of the condition known as benign prostate hyperplasia or BPH. Therefore, it is unlikely that the significant increase of prostate weight in rats treated with bee bread may cause prostate cancer or BPH.

In the present study, sperm counts were higher in rats treated with bee bread compared to control group. The percentage of abnormal sperms were lower in rats treated with bee bread compared to control group. Unfortunately, both comparison was not statistically significant. Previous study has shown that rats fed with pollen of *Raphanus. spp* and *Cistus spp*. Significantly increased the sperm count compared to control group (Selmano□ lu, 2009). Based on a study done by Mehraban et al. (2014), rats given with date palm pollen with the dose 120 and 240 mg/kg for 35 days significantly increase the sperm count and sperm motility compared to the control group. Rasekh et. al., (2015) has reported that orally administered Date Palm Pollen (DPP) the palm pollen on sperm parameters of infertile man by orally administered Date Palm Pollen (DPP) on infertile adult men significantly increased sperm motility, morphology and forward progressive motility. Bee pollen has at least 22 amino acids, which help in increasing the male infertility including arginine, L-carnitine, glutathione (Jessica, 2015). L-carnitine is amino acid is made in the body from the amino acids lysine and methionine and both found abundantly in bee pollen. L-carnitine plays a key role in the maturation of the sperm membrane and is crucial for sperm cells to function normally. Glutathione is not an amino acid but it is biosynthesised from the amino acids glycine, glutamic acid, and cysteine. Glutathione helps protect sperm by reducing oxidative stress and several studies have shown that it has a positive effect on sperm concentration, sperm morphology and motility (Gangwar et. al., 2018; Palani, 2018). Even though the finding on sperm count and sperm morphology were not

statistically significant but there is an increasing pattern in sperm count and reducing pattern in percentage of abnormal sperm in rats treated with bee bread compared to control group. Further study need to be done to elucidate this finding.

Table 1 Reproductive organ weights and sperm parameters of male rats in all experimental groups.

	Control	**Treatment**
Mean weight of testis (g/100 g b.w.)	0.59 ± 0.02	0.58 ± 0.01
Mean weight of epididymis (g/100 g b.w.)	0.16 ± 0.01	0.16 ± 0.01
Mean weight of seminal vesicle (g/100 g b.w.)	0.05 ± 0.01	0.06 ± 0.01
Mean weight of prostate gland (g/100 g b.w.)	0.07 ± 0.01	0.11 ± 0.01*
Sperm count (X 10^6)	25.50 ± 2.40	29.40 ± 3.60
Sperm morphology (%)	65.39 ± 1.88	54.53 ± 7.97

Data are presented as mean ± SEM (n = 6 in each group).

* $p<0.05$ compared with control group (Independent Samples T-test)

CONCLUSION

From the present result, it seems that bee bread supplementation does not caused any negative effect on the male reproductive system of rats. Bee bread supplementation may cause a positive effect on sperm count and sperm morphology since it increased the number of sperm and decreased the percentage of abnormal sperm. Further study need to be done to investigate more on the effect of bee bread on male reproductive system especially it's effect on reproductive hormones level.

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